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SYNTHESIS AND PRELIMINARY PHARMACOLOGICAL EVALUATION OF ANALOGUES OF CARACASANAMIDE, A HYPOTENSIVE NATURAL PRODUCT[1]

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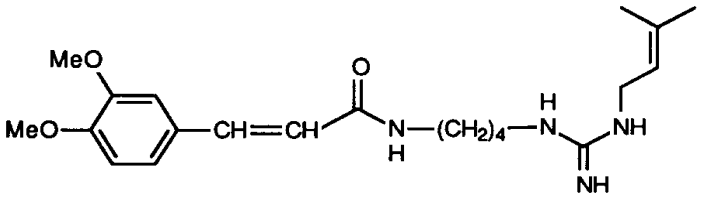
Abstract: Some analogues of the hypotensive agent caracasanamide have been synthesized and tested *in vivo* for cardiovascular effects. Derivative **2c** emerged as the most interesting compound in the series. Structure-activity relationship is also discussed.

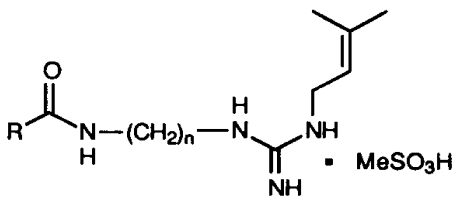
Synthetic guanidine derivatives have attracted pharmacologists in search of new antihypertensive drugs for their ability to block adrenergic nerve activity through central and/or peripheral mechanisms.[2,3] As a result, guanethidine,[4] guanabenz,[5] and guanfacine[6] have been introduced in antihypertensive drug therapy. We have recently reported[7] that the methanol extract of the Venezuelan plant *Verbesina caracasana* Fries yielded a series of active compounds, the least polar of which ($C_{21}H_{32}N_4O_2$) was named caracasanamide and assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]butane. The compound was a mixture of the (*E*)- and (*Z*)-forms and the pharmacological profile of the water-soluble (*Z*)-form (**1a**) and the synthesis of the (*E*)-form (**1b**) of caracasanamide have also been described.[8] Pharmacological studies indicated that caracasanamide is a hypotensive agent of low-mild potency, devoid of significant tachycardic effects, provided with central and peripheral mechanisms of action in affecting cardiovascular function, and with stimulating respiratory effects when administered at nontoxic doses. In the light of these results, we have synthesized analogues of caracasanamide with the aim of both developing new hypotensive agents, and gaining an insight into the structure-activity relationships in this series.

We report herein the synthesis and the preliminary pharmacological evaluation of the analogues **2a-f** of caracasanamide with a modified acyl group as well as two homologues **2g,h** with a longer (penta- or hexa-methylene) chain[9] between the 3,4-dimethoxycinnamoyl and the guanidino moieties (Chart 1). All the new compounds have been obtained and tested as the corresponding methanesulfonate salts.

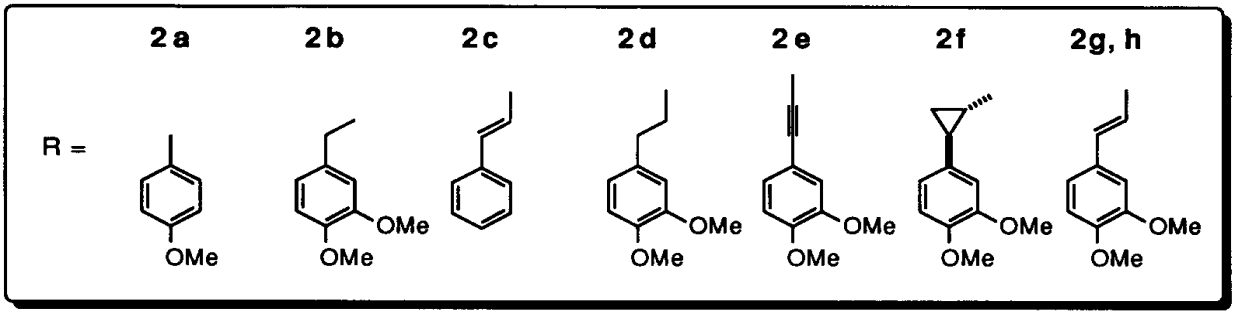
Chart 1.



1a (*Z*)-caracasanamide
1b (*E*)-caracasanamide



2a-f n = 4
2g n = 5
2h n = 6

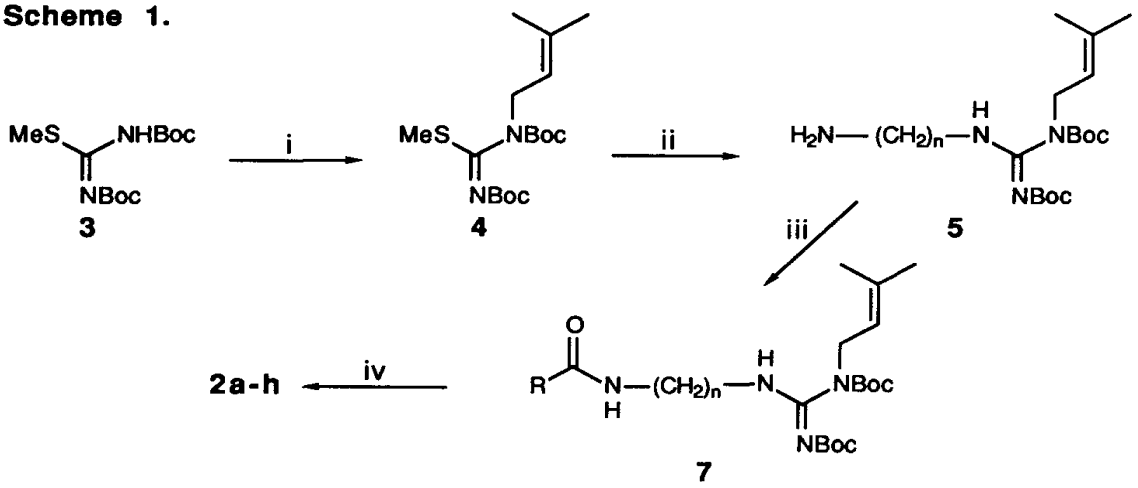


The synthesis of compounds **2a-h** is depicted in the Scheme 1. Phase transfer catalyzed alkylation of *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**3**)[8] with 4-bromo-2-methyl-2-butene gave *N,N'*-bis(*tert*-butoxycarbonyl)-*N*-(3-methyl-2-butenyl)-*S*-methylisothiourea (**4**) in 97% yield. On reaction with excess diaminoalkane, **4** afforded in 70-86% yield the intermediates **5** which were, in turn, acylated with the acyl chlorides **6**[10] to provide the Boc-protected compounds **7** (20-88% yield). Removal of the protective groups with methanesulfonic acid in refluxing 1,4-dioxane led to the target compounds **2a-h** as the corresponding salts (white foams) in 50-60% yield after chromatographic purification (SiO_2/chloroform:methanol 9:1).[11] Chemical and physical data of compounds **2a-h** are reported in Table 1.

The new compounds **2a-h** were tested for cardiovascular and respiratory effects, in comparison with natural caracasanamide (**1a**), through i.v. administration to adult male Wistar rats anaesthetized with sodium thiopental (50 mg/kg of body weight), at the dose of 4.12 μmol/kg corresponding to the ED_{50} for the hypotensive effect of **1a**. As for the natural compound, blood pressure (BP), heart rate (HR), maximum rate of

rise of the left ventricular isovolumetric pressure (dP/dt) as an index of cardiac inotropism,[12] and respiratory frequency (RF) were registered (Table 2).

Scheme 1.



Key: i) 4-bromo-2-methyl-2-butene, KOH, Bu_4NBr, CH_2Cl_2, H_2O; ii) $H_2N(CH_2)_nNH_2$, THF; iii) RCOCl (6), Et_3N, CH_2Cl_2; iv) $MeSO_3H$, 1,4-dioxane, reflux.
Boc = *tert*-butoxycarbonyl

Table 1. Chemical and physical data of compounds **2a-h**.

			FABMS (TDEG-Gly)	HRFABMS ($M^+ + 1$)	
compd	formula[a]	mol. weight[a]	*m/z*	found	required
2a	$C_{18}H_{28}N_4O_2$	332	333 ($M^+ + 1$)	333.2275	333.2291
2b	$C_{20}H_{32}N_4O_3$	376	377 ($M^+ + 1$)	377.2571	377.2553
2c	$C_{19}H_{28}N_4O$	328	329 ($M^+ + 1$)	329.2357	329.2341
2d	$C_{21}H_{34}N_4O_3$	390	391 ($M^+ + 1$)	391.2718	391.2709
2e	$C_{21}H_{30}N_4O_3$	386	387 ($M^+ + 1$)	387.2408	387.2396
2f	$C_{22}H_{34}N_4O_3$	402	403 ($M^+ + 1$)	403.2696	403.2709
2g	$C_{22}H_{34}N_4O_3$	402	403 ($M^+ + 1$)	403.2715	403.2709
2h	$C_{23}H_{36}N_4O_3$	416	417 ($M^+ + 1$)	417.2878	417.2866

[a]As the free base.

BP. All the synthetic analogues showed hypotensive activity. In particular, **2c**, **2f**, and **2g** caused higher hypotensive systolic responses than **1a**; **2h** was less active, while **2a**, **2b**, **2d**, and **2e** resulted to be equipotent to the natural caracasanamide. About the diastolic pressure values, **2h** was again less active than **1a**,

whereas **2a**, **2b**, **2c**, **2f**, and **2g** decreased the diastolic pressure more than **1a** (order of potency **2c>2g**, **2a>2b>2f**).

dP/dt. All the test compounds were able to increase cardiac inotropism, especially **2c** and **2g** which affected this function more than **1a**. On the contrary, **2d** and **2h** resulted less active.

HR. Derivatives **2c** and **2e** caused a slight tachycardic effect comparable to that of caracasanamide, compounds **2g** and **2h** being less effective. Bradycardia was observed after administration of **2a**, **2b**, **2d**, and **2f**.

RF. With the exception of **2g**, provided with a slight depressive effect on the respiratory frequency, all the tested compounds caused an increase of RF, although less marked than in the case of **1a**.

Table 2. Cardiovascular and respiratory effects of the test compounds **2a-h**, in comparison with **1a**, following i.v. administration at the dose[a] of 4.12 μmol/kg in anaesthetized male Wistar rats.

compd	blood pressure (BP) (ΔmmHg)		dP/dt_{max} (ΔmmHg/sec)	heart rate (HR) (Δbeats/min)	respiratory frequency (RF) Δbeats/min)
	systolic	diastolic			
2a	-26 ± 3	-35 ± 5*	+3249 ± 212	-16 ± 2*	+17 ± 3
2b	-22 ± 4	-31 ± 1*	+3088 ± 250	-24 ± 4*	+16 ± 4
2c	-47 ± 4*	-59 ± 6*	+6070 ± 474*	+19 ± 5	+12 ± 2*
2d	-15 ± 3	-14 ± 3	+2252 ± 173*	-12 ± 3*	+6 ± 4*
2e	-14 ± 4	-24 ± 5	+3424 ± 321	+14 ± 5	+14 ± 6
2f	-30 ± 1*	-26 ± 2*	+2781 ± 283	-19 ± 4*	+19 ± 1
2g	-34 ± 2*	-40 ± 4*	+5028 ± 116*	+8 ± 3*	-10 ± 2*
2h	-9 ± 2*	-8 ± 1*	+ 648 ± 33*	+4 ± 1*	+4 ± 2*
1a	-21 ± 3	-18 ± 2	+2996 ± 104	+22 ± 3	+23 ± 3

Values are means ± *S.E.M.* (n = 6). [a]Expressed as the free base. *p<0.05 (compared to natural caracasanamide).

On consideration of the above results, the following structure-activity relationships for the hypotensive agents of this class can be deduced.

i) The absence of the methoxy groups results in an increase of the hypotensive (both systolic and diastolic) activity as well as of cardiac inotropism.

ii) The one carbon atom lengthening of the alkyl chain (as in **2g**) has a positive influence on both BP and dP/dt, although causing a depressive respiratory effect; however, compound **2h**, having a longer chain, can be regarded as the least active product in the series.

iii) While the geometry of the double bond does not play a fundamental role, as (*E*)- and (*Z*)-caracasanamide show essentially the same activity,[8] conversely the presence of the double bond seems to be of major importance, since **1a**, **2c**, and **2g** are more active than compounds in which the double bond has been replaced by other structural features.

In conclusion, structural modifications of the caracasanamide skeleton allow for the modulation of the pharmacological profile of the natural product. Compound **2c** appears to be the most interesting derivative in our series and can be considered a hypotensive agent of low-mild potency, devoid of significant tachycardic effects and with stimulating respiratory effects when given at non toxic doses.[13]

Acknowledgment. We are grateful to the Italian MURST and to the University of Siena (60% funds) for financial support of this research.

References and Notes

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9. Studies on lower homologues of caracasanamide are in progress and will be reported in the due course.
10. Acid chlorides **6a-c** are commercially available, while **6d-g** were prepared by standard methods from the corresponding acids. 3,4-Dimethoxycinnamic acid and 3-(3',4'-dimethoxyphenyl)propionic acid were purchased from Aldrich Chemical Co., while 3-(3',4'-dimethoxyphenyl)propiolic acid and *trans*-2-

(3',4'-dimethoxyphenyl)cyclopropane-1-carboxylic acid were prepared as reported in the two following references, respectively: a) Adler, E.; Gustaffson, B. *Acta Chem. Scand.* **1963**, *17*, 27; b) Arvidsson, L.-E.; Johansson, A.M.; Hacksell, U.; Nilsson, J.L.G.; Svensson, K.; Hjorth, S.; Magnusson, T.; Carlsson, A.; Lindberg, P.; Andersson, B.; Sanchez, D.; Wikstrom, H.; Sundell, S. *J. Med. Chem.* **1988**, *31*, 92.

11. As an example we report herein the spectroscopic data of compound **2a**. IR ($CHCl_3$) ν_{max} 3240, 1730 cm^{-1}; ^1H NMR (CD_3OD) δ 1.55-1.70 (2s + m, 10H), 2.72 (s, 3H), 3.20-3.30 (m, 2H), 3.38-3.45 (m, 2H), 3.78-3.85 (s + d, 5H), 5.12-5.20 (t, 1H), 6.85-6.90 (d, 2H), 7.85-7.90 (d, 2H).
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